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At Date of Original Authorization	Period Covered	Nine Extended To	Allotment Rugger	Amount of California
11-Dec 1962			3125-1381.5902	20 63944
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Additional Authorizationa	Period Covered	Time Extended No	Allotzent Majora	Chlistion
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27 6 24. 00 11. 530.11 39.836.00 39,836.00 39.836.00 CONFACENCION DECUES. That payment CREDIT AMOUNT C/TSD/BB 71.80 AMOUNT MONEY ORDER 37 436.11 シャングアグ 7. OUTSTANDING ADVANCES END OF PERIOD (Arther Italing) A. CASH ON HAND END OF PERTOD OF BALANCE DUE ADVANCER Expenses - See attached DEB17 . VOUCHER NO. certification this accounting is true 11 DESCRIPTION OROUP 1 FXCLUDED PROM AUTOMATIC BOWN-ORALING AND BRUCKSSIFICATUOR HI CHECK TOTAL ACCOUNTED FOR 68.70 DUE DATE TOTALS 67-70 OBJECT CLASS TOTAL EXPENSES Z W Z OFFICE OF FINANCE COST - FAN ACCOUNT SYMBOL 22 October 1965 S. EXPENSES THIS PERIOD: SIGNATURE OF CERTIFYING OFFICER 6. REFUNDED MEREWITH DATE 950 CERTIFIED FOR PAYMENT OR CREDIT OF APPROVING SIDKEY COLLING. Sub-project # 143 VOLICHER 96 (When Filled In) 2011 1100 APPROVED GENERAL LEGGER ACCT NO. EXCLUSIVE USE 51.54 39.836.00 2 39,836.00 90 00 WF C00E 1 January 1963 SPACE BELOW FOR 42-47 METITIER SUBMITTED BY U O O O U \$19,836.00 \$20,000.00 FROM STATION DESCRIPTION OFFICER 2. OUTSTANDING ADVANCES BEGINNING OF PERIOD 1817.00C.NO. TOTAL TO ACCOUNT FOR I CERTIFY FUNDS ARE AVAILABLE DESCRIPTION-ALL OTHER ACCOUNTS 13-33 ACCOUNTING BY INDIVIDUAL CHARGE FAN ACCOUNT NO. NOTE: Follow Instructions on Reverse 3125-1390-3902 1125-1390-3902 FOR ADVANCE ADVANCE ACCOUNTS \$3-27 3. RECEIPTS THIS PERIOD! DATE FORM 282 esselere DESCRIPTION RECEIPT NUMBER 98 8 DOL 16.AEF DATE

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CERTIFICATION

This is to certify that I have received an accounting from MKULTRA Sub-project # 143 for the period I January 1963 - 22 October 1965. The accounting reflects expenses in the ancumt of \$39,823.29 against the remaining grant balance of \$39,836.00. The residual balance of \$12.71 should be written off as being impracticle for collection or detailed accounting.

The accounting statement and payment certification will be retained by TSD and will be made available for review in TSD if necessary.

I certify that satisfactory services represented by the accounting have been received and that to the best of my knowledge and belief the funds expended were for the purposes authorized by the project approval.







Statement of spechostures

Page 1 of 2

Expenditures 1/1/63-10/22/65

alaries	\$ 9,089,17
	650,00
	7,710,00
	400,00
	1,995.00
	250,60
	1,458.00
	2,150.00
	2,180.00
	200.00
	75.00
	26,157.17
rotal Salaries	
Consumable Supplies	8.34
FICA	5.55
Telephone & Telegren	575.43
Tubes & pipettes	1,066.35
Misc. Supplies	115.93
Electrical Supplies	176.07
Chemicala	15.33
Preight bills	113.16
Filter apparatus	599.71
Cultures :	357.99
Flasks, stoppers, syringes, etc.	89.65
Repair on Vacuum pump	59.50
mane sube mixer	120.00
Somice on a Sartorius Balance	3,303.11
Notal Consumable Supplies	2,303,22
Travel	
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Continued on next page.

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No.

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Cost Account 4125-1390-3902

Object Class

Date	Remarks and References	Obligations Incurred	Obligations Liquidated	Unliquidated . Balance
1/30/64	MKULTRA SUR 143	20,000.00		20,000 00
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Advance tour for funding KULTRA Subproject 1132, invoice # 2 paid to activity by approved by C/TSD on 25 January 1964. Accounting to be in accordance with KULTRA Fiscal Annex attachment B. Please forward checks (2) to C/TSD/BR thru TSD/BR 101 invoice # activity of protein for the fine for		attached pa	ingt	-		7	
Advance too Tribine Accounting to be in accordance with MNULTRA approved by C/TSD on 25 January 1964, Accounting to be in accordance with MNULTRA approved by C/TSD on 25 January 1964, Accounting to be in accordance with MNULTRA Placed Annex attended by C/TSD on 25 January 1964, Accounting to be in accordance with MNULTRA Placed for accordance of accordance with MNULTRA Placed for accordance of accordance with MNULTRA Placed for accordance of accordance of accordance with MNULTRA Placed for accordance of accordance of accordance with MNULTRA Placed for accordance of acco	Advance to C/			φ'			
Please forcard checks (2) to C/TSD/DB thru TSD/DF to later than 10 February 1964. Please forcard checks (2) to C/TSD/DB thru TSD/DF to later than 10 February 1964. STATUS G OUTSTANDING ADVANCES STATUS G OUTSTANDING		44	XXULTRA y 1964.	\	44	rdance	ctivity was mouth
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8 January 1964 MEHORANDUM FOR MKULTRA, Subproject No. 143 SUBJECT Under the authority granted in the memorandum dated 13 April 1953. from the DCI to the DD/A, and the extension of this authority in subsequent memoranda, Subproject 143 has been approved, and \$20,800.00 of the over-all Project MNULTRA funds have been obligated to cover the subproject's expenses and should be charged to cost center 4125-1390-3902 SKOCHAN ARBAWAK Concur: Concur: APPROVED FOR OBLIGATION OF FUNDS: Chief, DO/P/TSD GROUP I Excluded from automatic downgrading Distribution: Orig & 1 - Addresses

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Cost Account _3125=1390=3992

Date	Remarks and References	Obligations Incurred	Obligations Liquidated	Unliquidated Balance
188552	MKULTRA Sub Proj. 113 - Auth #1	20,629.14		20,629.41
0 1962	June #1		20,629.44	
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	Date:_11	December 1962
	MEMORANDUM FOR: C/TSD/FASS	
	SUBJECT MKULTRA, Subproject No.	
		ization # 1 idum dated 13 April 1953.
	Under the authority granted in the memoran from the DCI to the DD/A, and the extension of this	
	memoranda, Subproject 143 has been approve	ed. and \$20,629.44
	of the over-all Project MKULTRA funds have been o	
	subproject's expenses and should be charged to cost	Center 2152-1220-2205
	Concu	
	Agst. Chief, 15Dfor	1/1
1	Concur:	
-		
	APPROVED FOR OBLIGATION 1 CERTIFY	THAT FUNDS ARE AVAILABLE
	OF FUNDS:	10/18 10-3902
	0.00	
	Date: 11 Des_1103	
	Distribution: Orig & I - Addressee	GROUP 1 Excluded from automatic downgrading and
N.	2 - TSD/	declassification

I acknowledge receipt of funds in the amount stated here-under to be used for the purpose stated and accounted for CREDIT envisor behavious on a Please forward the check to Calef, 130/Blological Branch Carough 130/Budget Officer by 3 Jeouary 1963/ RECEIPT FOR FUNDS ADVANCED 71.80 AMOUNT (Ajuo Advance to Militia Subproject 183 Involce # 1 for activity approved by C/13D on 17 December 1962. Accounting for this atymbos will be in accordance with attachment A of Militia Fiscal Annex. T-1 - 30 9 8 5 I agree that I will fully account for this advance by submission of vouchers and refund of so the reporting point stated and refund of sa to the reaction point stated and by the due date checked below. In the event of my failure to any unexpended belonde. It authorities deduction in a salary to effect antifement. VOUCHER NO. (Finance OUE DATE ARRIVAL AT DESTINATION េ SIGNATURE OF ADVANCEE MONTHLY - ON THE LAST WORKDAY OF EACH MONTH 200 19 8 36 acre 113 - 101 - 100 X 137500 VOUCHER NO. 7-12 TCTALS 793.44 (Tea) DATE 2 Z \$19,836.00 SPACE BELOW FOR EXCLUSIVE USE OF FINANCE DIMISION S T SIGNATURE OF APPROVING OFFICER AUTHORIZED CERTIFYING OFFICER ₹., CERTIFIED FOR PAYMENT OR CREDIT SILENT CONTING ACCT NO. 54.85 19 Documber '62 BUILDING REPORTING POINT . WEADOUARTERS APPROVED 5 5 . 0.00 % 219 7 FINANCE DIVISION DATE CODE <u>.</u> in. 7 See attached Payment Inst. REVIEWED BY DATE DATE 3000 24.34 24.39 24.30 26.00 金田 人 PROJECT NO. UNACCOUNTED BALANCE CHANGE ALLOTMENT NO. 3125-1300-3922 MAME OF ADVANCES OUTSTANDING ADVANCES I CERTIFY FUNDS ARE AVAILABLE DESCRIPTION-ALL OTHER ACCOUNTS 13-35 PAYABLE REQUEST ING OFF ICER AMOUNT SIGNATURE SIGNATURE OBLIGATION REFERENCE NO. REQUEST FOR ADVANCE ADVANCE ACCOUNTS 13-27 DESCRIPTION-B 2016 STATUS A SUCCE DATE FORM 281 \$ X N 1 DATE •1 ÷

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7 4 8 4 • . . 19,834.00 . . . Lecknowledge receipt of funds in the amount stated here-under to be used for the purpose stated and accounted for as shown above. CARDIT Lagree that I will fully account for this advance by submission of vouchers and refund of any unexpeaded balance to the reporting paint states to so account and refund ÷... THUCKE 71-80 the check to thist, 1520/Biological Branch through 1520/Sudget Officer by 3 January 1963; 20 768 61 (Ajuo een AMOUNT Advance to MULLISA Embyroject 143 Invoice # 1 for ectivity approved by C/130 on 17 December 1962. Accounting for this edvance will be in accordance with attachment A of MULLISA Flecal Annex. - 1 - 7 0 0 の い . DUE DATE VOUCHER NO. (Finance ON OR ABOUT DESTINATION ON OR ABOUT ON THE LAST WORKDAY OF EACH MONTH (Co SIGNATURE OF ADVANCEE any unexpended balance. I muchorize deduction from my entary to affect entite. DEJECT CLASS • TOTALS 3902 X REF. BO. CHER NO DATE ALLOT OR CO ò SPACE BELOW FOR EXCLUSIVE USE OF FINANCE DIVISION . \$199836.00 \$ 793.44 (1 TELEPHONE EXT. 200 L 4 139 STUREY COTTLES AC/TED SIGNATURE OF APPROVING OFFICER AUTHORIZED CERTIFYING OFFICER CERTIFIED FOR PAYMENT OR CREDIT 3 THE STATE OF THE STATE OF ---ACCT. NO. 19 December '62 BUILDING REPORT ING POINT Filled In) FINANCE OIVESTON - MEADOUARTERS APPROVED 55 8 M 44 M DOE PREVIOUS COLVIOUS 119 DATE C006 • <u>.</u> REVIEWED BY gae attached Payment Inst. EXPEND CODE DATE DATE CHARGE ALLOTMENT NO. . 4 UNIVECCULARIED BALANCE S. 18. 8. 18. 3125-1390-3902 OUTSTANDING ADVANCES I CERTIFY FUNDS ARE AVAILABLE PAYABLESTO REQUESTING OFFICERS NAME OF . AMOUNT SIGNATURE formerd SIGNATURE OBLIGATION REFERENCE NO. ADVANCE ACCOUNTS \$3.27. ST FOR ADVANCE OF FUNDS Ö. Pletine STATUS 2016 REQUEST FORM 281 . DATE DATE

20 A. 1286

743-70 February 10, 1966

Gentlemen:

a detailed statement of his grant from we have prepared the detailed statement which you require.

Please let us know if there is anything more you need.

Sincerely,

Research Accountant

accounting to BF on 23 Jul, 966

773=11

February 23d, 1966

account with you this morning and it was agreed that:

- I. You would again contact the contractor re the \$18,20 unexpended balance reflected in the accounting for the period ending August 31, 1962, and not carried forward to the next grant year;
- 2. the current grant period would be extended in time only to 30 June 1966, giving the grantee the opportunity to expend in the field the \$123.69 residue, as requested in the January 10th, 1966 letter from (the original of this letter is enclosed herewith).

Also enclosed is the original of the February 10th, which forwarded 1966 letter from the which forwarded this grantee's accounting for expenditures during the period this grantee's accounting for expenditures during the period January 1st, 1963 through October 22nd, 1965.

Thanks very much for your help in the first item.

Enclosure: (2)

19 January 1966

MEMORANDUM FOR : Chief, TSD/BB

SUBJECT

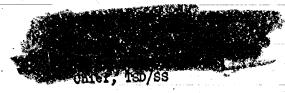
: Delinquent Accountings
MKULTRA, Subproject 143

1. Review of MKULTRA, Subproject 143 financial records indicate that accountings for grants are now past due. It is requested that your office initiate action to obtain the delinquent accountings.

2. For your information funds have been advanced to the grantee as follows.

Authorization 1 \$19,836.00 Grant period 1 Jan. - 31 Dec. 1963

Authorization 2 \$20,000.00 Grant period 1 Jan. - 31 Dec. 1964



Distribution:
Original & 1 - Addressee





Receipt is hereby acknowledged of deceipt is hereby acknowledged in the deceipt in the deceipt is hereby acknowledged in the deceipt in the deceipt is hereby acknowledged in the deceipt in the deceipt is hereby acknowledged in the deceipt drawn on the in the amount of \$20,000.00. hereby acknowledged of dated 7 February 1964. dorawn on the in the amount of \$800.00 PROJECT CRYPTO

MKULTRA #143

GRANTEE

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PRINCIPAL INVESTIGATOR

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DATE RENEWED

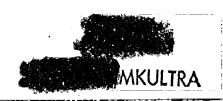
: 1 January 1964

During the past year, the investigators have studied a total of 57 compounds for their capacity to stimulate bacteria. These 57 compounds consisted of 34 phenols, 13 amines, 5 aldehydes and ketones, 3 quaternary ammonium compounds and 2 unclassified chemicals. This study has shown that the phenolic chemicals are the best compounds for stimulating bacteria. Of this group they have noted that Q-phenyl-phenol, p-tert-butyl-phenol and nonyl phenol are the best compounds studied so far.

They have been able to produce as much as a four-fold increase in microbial growth using these compounds. This degree of stimulation is not as great as they had hoped it would be. For this reason, during the past three weeks they changed their line of approach to the problem.

They have produced a product consisting of phenol, casein hydrolyzate, yeast extract and peptone which has a much greater stimulating capacity. It appears that concentrations as small as 50 ppm will give them a consistant 10 fold increase in growth. The deterioration of the cutting fluid is greater in three days than they have ever observed in their studies with these products at any time. In addition, as yet unpublished information from this laboratory indicates that this produce should neutralize practically any known biocide that might be added to cutting fluids.

It is believed that they have arrived at a point where dynamic progress can be anticipated. In view of the high proudcitivity and promise of results that will be of direct benefit to the operational aspects of DDP. It will be the purpose of the renewed project to combine the two principles of bacterial stimulation (the use of subeffective concentrations of inhibitors and the use of non-specific protective compounds) to determine the ultimate stimulation to be expected in cutting fluids. More emphasis will be given to actual degradative processes rather than stimulation of growth per se. In addition that agreed to investigate microbial fouling of diesel cooling systems in conjunction with his other work.



(When correct in)

	ich activity was with EKULTRA	1964.	mecount and ref	314		3		RECEIPT FOR FUNDS ADVANCED	perpose stated and accounted for	T & NOOMA					71-80 AMOUNT			Section 1		
000	# 2 whi	than 10 February 1964	for this advance by submission of vouchers and refund of any the dus date checked below. In the eventlofay fallure to so deduction from ay salary to effect settlement.	300		ON ARRIVAL AT DESTINATION	WORKDAY OF EACH MONTH	RECEIPT FOR	A sektowisoffe receipt of three in the auditorial and a second and a s		SIGNATURE OF ABVANCES		10v	VOUCHER NO. 7-12	150	262.67 C62.67 C62.67			TOTALS	O U.S. GOVERNMENT PROVINGE OFFICE: 1982
6.2 \$20,000,00 o Telephone ext.	143, Anvo	no later th	e that I will fully account for this advance by submission of vouchers and reporting point stated and by the due date checked below. In the event of my expended balance, I suthorize deduction from my malary to affect settlements						APPROVING OFFICER	TER DC	S FOR PAYMENT OR CREDIT AUTHORIZED CENTIFYING OFFICER		E OF FINANCE DIVISION		58-167 ALLOT OR C	ERAL GER NO.				
30 January 1964	L .	thru TSD/B	stil fully account in point in point state of the balance, I suchorize	REPORTING POINT	ON - MEADOUARTERS			 APPROVED	SIGNATURE OF A	SIDNEY COUNTER	CERTIFIED FOR PAYMENT OR		SPACE BELOW FOR EXCLUSIVE USE		47.52 OBLIG. 53	######################################				
1 m		to C/TSD/38		20	FINANCE DIVISION			-	3 Ma	9I.	DATE	T	SPACE BELOW	REVIEWED BY	40.42 43	CODE	\$			
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	COVERT ACTION SUPPORT	
Branch BB Category	Vahatana	(IVA
Project Title		nclassified (Data)
Project Crypto KKULTRA 143	Crypto Classification	Unclassified
Branch Project No. N.A.	Project Engineer	
Contractor		· · · · · · · · · · · · · · · · · · ·
Contract No. N.A.	Task No	N.A.
Type of Contract KKULTRA	Date Initiated	January 1964
FY 63 - \$20,629.4		
Cost FY 64 - \$20,800.0	O Completion Date	A V A CONTRACTOR OF THE CONTRA

Purpose: The objective of the first year was achieved. An approach to the stimulation of soluble-oil degradation was proved feasible. In addition a system for negating microbial inhibitors was developed. These two accomplishments appear to be the first significant breakthrough for microbial degradation of materials. It is planned to study further the limitations of these approaches and their application to a variety of practical situations during the coming year.

Status: Performance has been excellent. Has progressed beyond expectations. Current in fiscal matters.

Requirement: Generated by undocumented requests from DDP units and divisions as well as necessity for TSD to maintain a quick delivery capability to meet anticipated future operational needs.



The cost of this program for the second year is \$20,000.00. To this sum must be added \$800.00 representing a four per cent service charge to the The will function as cut-out and cover for this grant. The cover title of the project is Total cost of the project will not exceed \$20,800.00 Charges should be made against Allotment No. 4125-1390-3902. will request the to submit a summary accounting of monies received and to return any unexpended grant funds at the end of the project year. 7. Title to any permanent equipment purchased by funds granted the shall be retained by them in lieu of higher overhead rates. 8. It was mutually agreed that documentation and accounting for travel expenses which are reimbursable by the hall conform with the accepted practices of that institution. SD/Biological Branch Attachments: Note: Due date for accounting of Proposal & budget previous year's funds is 31 march 1914.

MKULTRA

Distribution:

Original only

DRAFT

8 January 1964

MEMORANDUM FOR : THE RECORD

SUBJECT

MKULTRA, Subproject 143

1. The purpose of subproject 143 is to continue support

on an approach to microbial

deterioration of materials.

- 2. During the first year a system was developed which allows a 10 fold stimulation of the microbial deterioration of soluble oil coalants (as a type system) using extremely small quantities of active material. In addition a system was found which apparently serves to inactivate most microbial inhibitors in such a product. This was much more of an accomplishment than was expected. For the coming year it is planned to explore the limitations of these approaches as they apply to cutting oils and other petroleum based products. It is hoped that these techniques will be applicable across—the-board to all sabotage or harassment systems involving microorganisms, promoting a more deliverable package to the operator.
- grincipal investigator, has been cleared through SECRET.



in :: 8 January 1964

MENORANDUM FOR : CITSDEFASS

Subject

MKULTRA, Subproject Fo. 143

Under the authority granted in the sescrandum dated 13 April 1953. from the DCI to the DD/A, and the extension of this authority in subsequent memoranda, Supproject 143 has been approved, and \$20,800,00 of the over-all Project MAULTEA funds have been obligated to cover the subproject's expenses and should be charged to cost center 4125-1390-3902





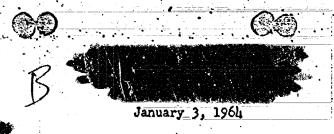
APPROVED FOR OBLIGATION OF FUNDS Date:

Distribution:

Orig & 1 - Addressee

- TSD. BB.

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As you know, the grant from the one year old and it is time to consider renewal of the grant.

During the past year we have studied a total of 57 compounds for their capacity to stimulate bacteria. These 57 compounds consisted of 34 phenols, 13 amines, 5 aldehydes and ketones, 3 quaternary ammonium compounds and 2 unclassified chemicals. This study has shown that the phenolic chemicals are the best compounds for stimulating bacteria. Of this group we have noted that Q-phenyl-phenol, p-test-butylphenol, disec-butylphenol, dodecyl pehnol, 2,4-dimethyl-6-test-butylphenol and nonyl phenol are the best compounds studied so far.

We have been able to produce as much as a four-fold increase in microbial growth using these compounds. This degree of stimulation is not as great as we had hoped it would be. For this reason, during the past three weeks we changed our line of approach to the problem.

We have produced a product consisting of phenol, casein, hydrolyzate, yeast extract and peptone which has a much greater stimulating capacity. It appears that concentrations as small as 50 ppm will give us a consistant 10 fold increase in growth. The deterioration of the cutting fluid is greater in three days than we have ever observed in our studies with these products at any time. In addition, as yet unpublished information from this laboratory indicates that this product should neutralize practically any known biocide or stat that might be added to cutting fluids.

We feel that we now have arrived at a point where we can make some dynamic progress. During the coming year we hope to:

- 1. Study additional chemicals and media products for their stimulatory capacity.
- 2. Determine the best ratios of the media components and generally improve the formulation.
- 3. Determine the effect of the formulation on other petroleum products.
- 4. Determine if the formulation has the capacity to neutralize antimicrobial agents.
- 5. Begin work on how the formulation stimulates microorganisms.
- 6. Determine the effect of the formulation plus urine on microbial growth.

I believe these points cover the main objectives for the coming year. Kindest personal regards.

Sincerely yours,







PROPOSED GRANT COST

A٠	Principal Investigator (time)		3990.00	
	Full time research assistants		9417.00	<u>.</u>
В.	Expendable equipment and supplies		2560.00	<u> </u>
c.	Typing reports, papers, etc.		700.00	
D.	Overhead (20%)		3333.00	
	TOTAL	20	0,000.00	

143-20

27 January 1964

MEMORANDUM FOR THE RECORD

SUBJECT: MKULTRA Subproject 143

- 1. At the request of both Chief and Deputy Chief, TSD was contacted for the purpose of funding this MKULTRA Subproject through one of the established funding mechanisms controlled by Due pri marily to the funding date, it was not possible to accomplish this at this time.
- 2. Arrangements will be made with to project our funding requirements for MKULTRA Subprojects on a semi-annual basis. With this lead time, feels that they may be capable of funding our total requirements.
- 3. will meet with to discuss other Subprojects funding mechanisms at 1115 this date.

Chief, TSD/Support Staff

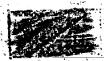
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	MKULTRA	Data Initiated	1 January
Type of Contrac	EAUDINA	Date Initiated	
Coct \$2	0,629.44	Completion Date	l year

Purpose: To investigate the potential of a new approach to the stimulation of microbial deterioration of petroleum products and its possible application to other degredative processes

Status: Contractor has developed a model system for desired system.
Will now attempt to apply it on small scale. Current in
fiscal matters.

Requirement: Generated by undocumented requests from DDP units and divisions as well as necessity for TSD to maintain a quick delivery capability to meet anticipated future operational needs.



143-22

RECEIPT

Receipt is hereby acknowledged of the dated January 7, 1963, drawn on the payable to in the amount of \$19,836.00

Receipt is hereby acknowledged of lated January 7, 1963, dwywn on the payable to in the amount of \$195.14

Date Jan 10 1963

0 (43.23

DRAFT

11 December 1962

MEMO FOR : THE RECORD

SUBJECT : MKULTRA, Subproject 143

1. The purpose of subproject 143 is to support the work of

on an approach to the stimulation of petroleum product deterioration.

- 2. Although the model system for these studies will be petroleum products, this appraoch to stimulation of deterioration may
 have wide application to all areas of biological sabotage currently
 of interest to TSD.
- 3. principal investigator, has been cleared through SECRET.
- 4. The cost of this program for the first year is \$19,836.00.

 To this sum must be added \$793.44 representing a four per cent service charge to the will function as cut out and cover for this grant. The cover title of the project is
- 5. Total cost of the project for a period of one year will not exceed \$20,629.44. Charges should be made against Allotment No.

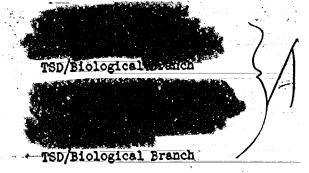
3125-1390-3992.

6 vill request the to submit

a summary accounting of monies received and to return any unexpended grant funds at the end of the project year.

7. Title to any permanent equipment purchased by funds granted the shall be retained by them in lieu of higher over-head rates.

- 8. It was mutually agreed that documentation and accounting for travel expenses which are reimbursable by the shall conform with the accepted practices of that institution.
- 9. The requirement for a six months' informal accounting on the part of the principal investigator is waived.



Attachment: Proposal & Budget

Distribution:
Original only



149.23

GRANT REQUEST

from -



CAB

Brographical Sketch

Entire page

Publications and Papers

5 pages

Symposia and Books

1 page

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OBJECTIVE OF THIS INVESTIGATION

To determine if the growth of microorganisms in petroleum products can be markedly increased by the addition of substatic concentrations of antimicrobial agents.

LITERATURE REVIEW

The literature has been carefully searched to determine what is known concerning the use of antimicrobial agents to stimulate microbial growth. To date, only two papers have been found that relate to this topic.

Imshenetskil and Perova (Institute of Microbiology, Academy of Science U.S.S.R., Mikrobiologiya, 23, 53-56, 1954) found that cultures of Saccharomyces cerevisiae trained to grow in the presence of small concentrations of HgCl2 or phenols have much higher fermentation rates than cultures that have not been trained to grow in the presence of these compounds. As little as 50 ppm of HgCl2 accelerates the production of beer. These workers believe that this effect is due to traces of inhibitor in the environment and not because of some change in the physiology of the cells.

reported that 50 ppm of 2,4,6-trichloro phenol could_markedly stimulate the growth of bacteria_in_emulsion_oils. In some instances_this______compound produced_slmost_a_three fold_increase in the number_of cells______in the environment.______

found that 25 to 150 ppm of 2,4-dinitrophenol increases the oxidation of C4, C6, C8, C10, C12, C13, C14, and 66 fatty acids. The increase was as much as 100% and was not associated with an increase in

the number of cells in the environment.

These results imply that two types of stimulation may be produced by substatic concentrations of inhibitors. First, a metabolic stimulation may be produced where the cells do not increase in number but this metabolic rate is increased. Second, the number of cells in the environment may be increased by the compound. It remains to be seen whether there is a stimulation in the individual metabolic activity of each cell in this case. There is an increase in activity, however, this may only be due to the greater number of cells.

UNPUBLISHED DATA

have found that 1000 ppm of 2-nitro-1-butyl acetate produce as 3 fold increase in the production of penicillin by Penicillium chrysogenum.

PLAN OF ATTACK

be limited by the rate of dufision of the compound across the permeability barrier. It is also well known that surface active agents increase the permeability of the cytoplasmic membrane. Several years ago developed the idea that the use of surface active compounds may increase the accessibility of hydrocarbon substrates to microbial attack and thus speed up the oxidation of these compounds.

Due to several factors including a lack of funds, this idea has never been tested to any extent. To date, we have studied 3 phenolic compounds. Two of these compounds produced stimulation and one did not.

We propose to study the effect of substatic concentrations of

phenois, fatty amines/alcohols and quatemary ammonium compounds on the oxidation of two different synthetic coolants and one petroleum type product. We may also use other antimicrobial agents other than those listed above.

EXPERIMENTAL PROCEDURE

CULTURES

Pure cultures of microorganisms belonging to the Genus

Pseudomonas will be used in this investigation. The cultures

will be isolated from samples of used cutting fluids obtained in
the Houston area.

MEDIA

Whenever possible, the organisms will be maintained in cutting fluids. In some instances conventional media such as nutrient broth, meat infusion broth, or an inorganic salts medium may be used in this investigation.

INCUBATION TEMPERATURE

All experiments will be done at 30°C. The length of the incubation period will vary according to the organism and the particular experiment.

CHEMICALS

The best grade of chemical obtainable will be used throughout this investigation.

GLASSWARE

All flasks, tubes and other glass equipment will be acid cleaned prior to each experiment.

HYDROGEN ION CONCENTRATION

The pH of the environment will vary according to the particular experiment. In general, we will let the Ph of the cutting fluid

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determine the pH of the experiment.

INOCULUM

The cells used in the experiments will be grown on an appropriate medium. They will be harvested after a suitable incubation period and washed three times in isotonic saline. The cells will be suspended in isotonic saline and uniform quantities of this suspension will be used to inoculate the tubes or flasks.

STERILITY

We will employ suitable procedures for sterilizing the media containing the petroleum products.

CONTROLS:

Appropriate controls will be included in each experiment. Each experiment will be done in duplicate and repeated at least once.

CUTTING FLUIDS

Three different cutting fluids will be used in this investigation consisting of two chemical coolants and one petroleum product.

EXPERIMENTAL TECHNIQUE

Each cutting fluid will be prepared in a 11 - 25 dilution in tap water. One hundred ml of this coolant will be placed in each 250 ml Erlenmeyer flask. The number of flasks used in each experiment will vary from time to time. The inhibitor will be added to the series of flasks in varying concentrations. These flasks will be sterilized by autoclaving and then inoculated with the test organisms. The flasks will be incubated for a period of time (probably two weeks) in a shaking incubator.

At periodic intervals of about 2 days time the number of cells

per ml will be determined for each flask. At the same time the pH

of the flask will be determined and other biochemical tests (such as ,

dehydrogenase activity) may be conducted to determine the degree of

metabolic activity in the environment. At the end of the experiment

the remaining cutting fluid will be poured into a volumetric cylinder to determine the quantity of free oil in the product (this test will be used on the petroleum coolant only). The quantity of free oil present is an excellent index of the degree of attack on the emulsifier.

In addition, Warburg studies will be conducted at the optimim stimulatory concentration determined, in the growth expedent to establish the effect of the antimicrobial agent on oxygen uptake.

We will study as many compounds as possible during the year period of this grant. In no case will this be less than twenty-five compounds.

COST OF PROJECT

A. Principal Investigator \$200.00/month (9 months)	\$ 1,800.00
Full time salary to work on project in Summer	3,210.00
2 Full Time Research Assistants (\$375/month each)	9,000.00
8. Permanent Equipment	\$ 14,010.00
I Shaking incubator	\$ 1,080.00
C. Expendable Equipment & Supplies	\$ 900.00
D. Publication Cost	
Typing report, papers, and purchasing reprints of publications that may arise from this work	\$ 400.00
	\$ 16,390.00
E. Overhead (20%)	\$ 3,446.00
	\$ 19,836.00